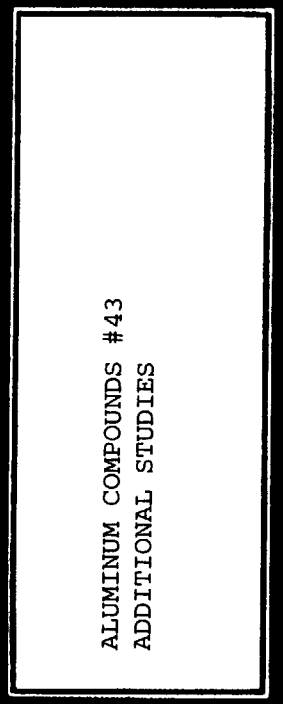
Aluminum Compounds-Additional Studies #43 5/14/73



Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVAIR IN FEMALE ALBINO RATS

MAY 14, 1973

IBT NO. B2423B

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

TOXICOLOGY
ENVIRONMENTAL SCIENCES
CHEMISTRY
PLANT SCIENCES
MEDICAL SCIENCES

AREA CODE 312
TELEPHONE 272-3030

May 14, 1973

Mr. R. S. Bryant
Manager, Quality Assurance
Industrial Chemical Division
Stauffer Chemical Company
Westport, Connecticut 06880

Dear Mry Bryant:

Re: IBT No. B2523B - 90-Day Subacute Oral Toxicity Study with Levair in Female Albino Rats

We are submitting herwith our laboratory report dated May 14, 1973, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/mp

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVAIR IN FEMALE ALBINO RATS

MAY 14, 1973

IBT NO. B2423B

I. Introduction

A sample identified as Levair was received from the Stauffer Chemical Company for the purpose of conducting a 90-day subacute oral toxicity study using female albino rats as test animals. The following report presents the results of this investigation.

II. Summary

A 90-day subacute oral toxicity study was conducted with groups of female albino rats fed Levair at dietary levels of 300 and 1,000 ppm. Microscopic examination of the kidneys revealed microconcretions in both the control and test animals. However, a greater incidence was observed in the test groups (60% in T-I and 70% in T-II) as compared to the control group (40%). No abnormalities were observed in the survival, growth rate or kidney weights in any of the groups tested.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: M. S. Reyna

M. S. Reyna, B. S.
Group Leader
Rat Toxicity

Report approved by: Gerald L Kennedy Jr

Gerald L. Kennedy, Jr., B. S.
Section Head, Toxicology

M. L. Keplinger

M. L. Keplinger, Ph. D.
Manager, Toxicology

msh:psh

III. Procedure

A. Experimental Animals

The animals employed in the study were Charles River strain* albino rats. Forty-five female rats were selected for the experiment and housed individually in standard, wire-bottomed, steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment and individual animal number.

B. Organization of Groups

A structural outline of the experiment is shown in Table I.

TABLE I

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Outline of Experiment

Group	Number of Animals	Dietary Level (ppm)
Control	15	None
T-I	15	300
T-II	15	1,000

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

C. Body Weights

Each animal used in the study was weighed on the first day of the test and at monthly intervals thereafter. The weights were recorded and served as an index to growth. Weight gains were computed at the conclusion of the 90-day test period.

D. Diet Preparation

The diet for any given group was prepared by blending the appropriate amount of Levair with standard rat ration in a Hobart Mixer.

Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for one week's ad libitum feeding, However, checks were made periodically to ensure that the food jars were not empty.

E. Mortality and Reactions

Abnormal reactions and deaths were recorded daily during the investigation.

F. Pathologic Studies

Following 90 days of feeding, all surviving rats were sacrificed by carbon dioxide asphyxiation and autopsied. Animals which died during the study were examined grossly unless examination was precluded by postmortem autolysis. At the time of the final sacrifice the kidneys from each rat were removed and preserved in formalin solution. Also at autopsy the weights of the kidneys were determined and recorded.

Microscopic examination of the kidneys taken from 10 rats of the control, T-I and T-II groups was conducted. The kidneys were stained with Hematoxylin-Eosin.

G. Kidney Weights and Kidney to Body Weight Ratios

Statistical analyses were conducted upon the absolute kidney weights and their corresponding ratios to the weight of the body. An Analysis of Variance was conducted first and any significant effects disclosed by that treatment were further studied by Student's "t"-tests.

IV. Results

A. Body Weights

Body weight data collected during the 90-day test period are summarized in Table II. Also included in the table are 90-day average total weight gains.

Comparisons of final body weights and total weight gains revealed no significant differences between test and control rats.

TABLE II

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Body Weight and Total Weight Gain Data

Summary of Mean Values

Dietary Level (ppm)	Body Weight (g) Month: 0	1	2	3	3-Month Total Weight Gain (g/rat)
Control	122	231	285	302	180
300	122	233	290	302	180
1,000	122	222	280	303	181

B. Mortality and Reactions

One death occurred during the study. This death resulted from natural causes.

No untoward behavioral reactions were noted among any of the animals employed in the study.

C. Pathologic Studies

1. Gross Pathologic Findings

No outstanding differences were noted between test and control rats upon gross pathological examination.

2. Kidney Weight and Kidney to Body Weight Ratio Data

The results of the statistical analyses conducted on absolute kidney weights and kidney to body weight ratios are summarized in Table III. The individual kidney weights are listed in Table IV.

Statistical analysis of the kidney to body weight ratios revealed an effect at the 95% confidence level in the T-II (1,000 ppm) group. This effect appeared to be caused by an extremely small standard deviation and is of no toxicological significance. Absolute kidney weights revealed no significant differences between the control and test groups.

TABLE III

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - KIDNEYS

DIETARY LEVEL (PPM)	ORGAN WEIGHT (GM) MALES	ORGAN WEIGHT (GM) FEMALES	ORGAN/BODY WEIGHT RATI (GM/100 GM) MALES	ORGAN/BODY WEIGHT RATI (GM/100 GM) FEMALES
NONE	0.000	2.245	0.0000	0.7457
300	0.000	2.400	0.0000	0.8017
1000	0.000	2.168	0.0000	0.6910*

NO STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE IV

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT DATA - KIDNEYS

FEMALES

GROUP	DIETARY LEVEL (PPM)	RAT NUMBER	ORGAN WEIGHT (GM)	ORGAN/BODY WEIGHT RATIO (GM/100 GM)
C	NONE	1	1.780	0.71200
		2	2.430	0.69034
		3	2.280	0.76254
		4	2.150	0.82061
		5	2.540	0.73837
		7	2.050	0.68561
		8	2.340	0.70694
		9	2.140	0.67936
		10	2.340	0.87969
		11	2.380	0.76527
		12	2.140	0.73539
		13	2.420	0.74461
		14	2.310	0.72413
		15	2.140	0.79553
T-I	300	16	1.910	0.72348
		17	2.540	0.84949
		18	2.320	0.71604
		19	2.470	0.73293
		20	2.110	0.79924
		21	2.390	0.75873
		22	2.480	0.79999
		23	2.450	0.71637
		24	2.650	0.87458
		25	2.430	0.80198
		26	2.210	0.69062
		27	2.250	0.77054
		28	2.060	0.66451
		29	2.190	0.72999
		30	3.550	1.39763

TABLE IV continued

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT DATA - KIDNEYS

FEMALES

GROUP	DIETARY LEVEL (PPM)	RAT NUMBER	ORGAN WEIGHT (GM)	ORGAN/BODY WEIGHT RATIO (GM/100 GM)
T-II	1000	61	2.000	0.65573
		62	2.030	0.58840
		63	2.080	0.68674
		64	1.850	0.59870
		65	2.250	0.68181
		66	2.320	0.81403
		67	2.100	0.70000
		68	2.600	0.79268
		69	2.340	0.76221
		70	2.260	0.77133
		71	1.950	0.61904
		72	2.030	0.67892
		73	2.150	0.70957
		74	2.240	0.66076
		75	2.330	0.64364

3. Histopathologic Findings

Histopathologic examination of the kidneys from 10 rats in the control, T-I, and T-II groups was conducted. A greater incidence of microconcretions in the test groups (T-I 60%, T-II 70%) was observed than in the control group (40%). Tables V through VII list all histopathologic changes noted.

The pathologist's statement follows on the next page.

IBT No. B2423B
Stauffer Chemical Company

I have completed a histopathologic evaluation of sections of both kidneys from rats of Study Number B2423B. There are calcified microconcretions present in the lumens of some renal tubules located at the corticomedullary junction of both control and test animals. However, the number of affected animals is greater among the animals fed Levair. The microconcretions also tend to be more numerous and they are slightly larger in some of these animals. Small isolated microconcretions are occasionally observed in the renal tubules of untreated control rats and they are regarded as lesions of naturally occurring disease. In the present study, the formation of such microconcretions appears to have been exacerbated by the test material. The other lesions described in the kidney are those of naturally occurring disease.

D. E. Gordon

Donovan E. Gordon, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists

TABLE V

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: Control

	An. No. 1	An. No. 2	An. No. 3	An. No. 4	An. No. 5
Kidney		Mc[1]/b	Mc[1]/b, FLI[1]		Mc[1]/b, FLI[1]

TABLE V continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: Control

	An. No. 7	An. No. 8	An. No. 9	An. No. 10	An. No. 11
Kidney		Mc^3/b			

Grading System:

+1 = Minimal in severity (less than 10 microconcretions/section)
+2 = Mild in severity (10-30 microconcretions/section)
+3 = Moderate in severity (30-50 microconcretions/section)
+4 = Marked in severity (50-75 microconcretions/section)

Codes:

Mc = Calcified microconcretions in tubules
/b = Bilateral
FLI = Focal lymphoid infiltration

TABLE VI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-I (300 ppm)

	An. No. 16	An. No. 17	An. No. 18	An. No. 19	An. No. 20
Kidney	Mc[1-2]/b, FLI		Mc[1]/b		FLI[1]

TABLE VI continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-I (300 ppm)

	An. No. 21	An. No. 22	An. No. 23	An. No. 24	An. No. 27
Kidney	Mc ^{1}/b	Mc ^{2}/b, FLI ^{1}	Mc $^{1-2}$/b	Mc ^{1}/u, FLI ^{1}	

Grading System:

+1 = Minimal in severity (less than 10 microconcretions/section)
+2 = Mild in severity (10-30 microconcretions/section)
+3 = Moderate in severity (30-50 microconcretions/section)
+4 = Marked in severity (50-75 microconcretions/section)

Codes:

Mc = Calcified microconcretions in tubules
/b = Bilateral
/u = Unilateral
FLI = Focal lymphoid infiltration

TABLE VII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-II (1,000 ppm)

	An. No. 31	An. No. 32	An. No. 33	An. No. 34	An. No. 35
Kidney	Mc^{1-2}/b, FLI^{1}	Mc^{1}/b	Mc^{1-2}/b	Mc^{1}/b	Mc^{2-3}/b

TABLE VII continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-II (1,000 ppm)

	An. No. 36	An. No. 37	An. No. 38	An. No. 39	An. No. 40
Kidney			Mc [1]/u	Mc [1]/b	

Grading System:

+1 = Minimal in severity (less than 10 microconcretions/section)
+2 = Mild in severity (10-30 microconcretions/section)
+3 = Moderate in severity (30-50 microconcretions/section)
+4 = Marked in severity (50-75 microconcretions/section)

Codes:

Mc = Calcified microconcretions in tubules
/b = Bilateral
/u = Unilateral
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Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH KASAL IN FEMALE ALBINO RATS

MAY 14, 1973

IBT NO. B2423A

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

AREA CODE 312
TELEPHONE 272-3030

TOXICOLOGY
ENVIRONMENTAL SCIENCES
CHEMISTRY
PLANT SCIENCES
MEDICAL SCIENCES

May 14, 1973

Mr. R. S. Bryant
Manager, Quality Assurance
Industrial Chemical Division
Stauffer Chemical Company
Westport, Connecticut 06880

Dear Mr. Bryant:

Re: IBT No. B2423A - 90-Day Subacute Oral Toxicity Study with Kasal in Female Albino Rats

We are submitting herewith our laboratory report dated May 14, 1973, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/mp

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH KASAL IN FEMALE ALBINO RATS

MAY 14, 1973

IBT NO. B2423A

I. Introduction

A sample identified as Kasal was received from the Stauffer Chemical Company for the purpose of conducting a 90-day subacute oral toxicity study using female albino rats as test animals. The following report presents the results of this investigation.

II. Summary

A 90-day subacute oral toxicity study was conducted with groups of female albino rats fed Kasal at dietary levels of 300 or 1,000 ppm. Microscopic examination revealed microconcretions in the kidneys of both the control and test rats. The incidence was greater in the test groups (80% for both T-I and T-II) than in the control group (40%). No abnormalities were observed in the survival, growth rate or kidney weights of any of the rats tested.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: M. S. Reyna

M. S. Reyna, B.S.
Group Leader
Rat Toxicity

Report approved by: Gerald L. Kennedy, Jr.

Gerald L. Kennedy, Jr., B.S.
Section Head, Toxicology

M. L. Keplinger

M. L. Keplinger, Ph.D.
Manager, Toxicology

chm:psh

III. Procedure

A. Experimental Animals

The animals employed in the study were Charles River strain* albino rats. Forty-five female rats were selected for the experiment and housed individually in standard, wire-bottomed, steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment and individual animal number.

B. Organization of Groups

A structural outline of the experiment is shown in Table I.

TABLE I

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Outline of Experiment

Group	Number of Animals	Dietary Level (ppm)
Control	15	None Administered
T-I	15	300
T-II	15	1,000

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

C. Body Weights

Each animal used in the study was weighed on the first day of the test and at monthly intervals thereafter. The weights were recorded and served as an index to growth. Weight gains were computed at the conclusion of the 90-day test period.

D. Diet Preparation

The diet for any given group was prepared by blending the appropriate amount of Kasal with standard rat ration in a Hobart Mixer.

Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for one week's ad libitum feeding. However, checks were made periodically to ensure that the food jars were not empty.

E. Mortality and Reactions

Abnormal reactions and deaths were recorded daily during the investigation.

F. Pathologic Studies

Following 90 days of feeding, all surviving rats were sacrificed by carbon dioxide asphyxiation and autopsied. Animals which died during the study were examined grossly unless examination was precluded by postmortem autolysis. At the time of the final sacrifice the kidneys from each rat were removed and preserved in formalin solution. Also at autopsy the weights of the kidneys of each rat were determined and recorded.

Microscopic examination of the kidneys taken from 10 rats of the control, T-I and T-II groups was conducted. The kidneys were stained with Hematoxylin-Eosin.

G. Kidney Weights and Kidney to Body Weight Ratios

Statistical analyses were conducted upon the absolute kidney weights and their corresponding ratios to the weight of the body. An Analysis of Variance was conducted first and any significant effects disclosed by that treatment were further studied by Student's "t"-tests.

IV. Results

A. Body Weights

Body weight data collected during the 90-day test period are summarized in Table II. Also included in the table are 90-day average total weight gains.

Comparisons of final body weights and total weight gains revealed no significant differences between test and control rats.

TABLE II

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Body Weight and Total Weight Gain Data

Summary of Mean Values

Dietary Level (ppm)	Body Weight (g) Month: 0	1	2	3	Total Weight Gain (g/rat)
Control	122	231	285	302	180
300	122	288	279	304	182
1,000	122	240	295	315	193

B. Mortality and Reactions

Two deaths occurred during the study. These deaths resulted from natural causes.

No untoward behavioral reactions were noted among any of the animals employed in the study.

C. Pathologic Studies

1. Gross Pathologic Findings

No outstanding differences were noted between test and control rats upon gross pathological examination.

2. Kidney Weight and Kidney to Body Weight Ratio Data

The results of the statistical analyses conducted on absolute kidney weights and kidney to body weight ratios are summarized in Table III. The individual kidney weights are listed on Table IV.

There were no statistically significant intergroup differences.

TABLE III

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - KIDNEYS

DIETARY LEVEL (PPM)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)	
	MALES	FEMALES	MALES	FEMALES
NONE	0.000	2.245	0.0000	0.7457
300	0.000	2.166	0.0000	0.7175
1000	0.000	2.247	0.0000	0.7227

NO STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE IV

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT DATA - KIDNEYS

FEMALES

GROUP	DIETARY LEVEL (PPM)	RAT NUMBER	ORGAN WEIGHT (GM)	ORGAN/BODY WEIGHT RATIO (GM/100 GM)
C	NONE	1	1.780	0.71200
		2	2.430	0.69034
		3	2.280	0.76254
		4	2.150	0.82061
		5	2.540	0.73837
		7	2.050	0.68561
		8	2.340	0.70694
		9	2.140	0.67936
		10	2.340	0.87969
		11	2.380	0.76527
		12	2.140	0.73539
		13	2.420	0.74461
		14	2.310	0.72413
		15	2.140	0.79553
T-I	300	31	2.210	0.71521
		32	2.130	0.74216
		33	2.250	0.76791
		34	2.070	0.71875
		35	1.950	0.73584
		36	2.390	0.80471
		37	1.760	0.68482
		38	2.140	0.74305
		39	2.420	0.77813
		40	2.190	0.73244
		41	2.130	0.67619
		42	2.190	0.61002
		43	2.270	0.64672
		44	2.280	0.71249
		45	2.120	0.69508

TABLE IV CONTINUED

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT DATA - KIDNEYS

FEMALES

GROUP	DIETARY LEVEL (PPM)	RAT NUMBER	ORGAN WEIGHT (GM)	ORGAN/BODY WEIGHT RATIO (GM/100 GM)
T-II	1000	76	2.380	0.74608
		77	2.030	0.72241
		78	2.270	0.69846
		80	2.180	0.71241
		81	2.350	0.72085
		82	2.210	0.68633
		84	2.140	0.70394
		85	2.540	0.72571
		86	2.150	0.77338
		87	2.060	0.69360
		88	2.120	0.67088
		89	2.540	0.81935

3. Histopathologic Findings

Histopathologic examination of the kidneys from 10 rats of the control, T-I and T-II groups was conducted. Microscopic examination of sections of kidney from the rats tested revealed an increase in the number of microconcretions in the test groups (80% in T-I and T-II as compared to 40% in the control group). Tables V through VII list all histopathologic changes noted.

The pathologist's statement follows on the next page.

IBT No. B2423A
Stauffer Chemical Company

I have completed a histopathologic evaluation of sections of both kidneys from rats of Study Number B2423A. There are calcified microconcretions present in the lumens of some renal tubules located at the corticomedullary junction of both control and test animals. However, the number of affected animals is greater among the animals fed Kasal. The microconcretions also tend to be more numerous and they are slightly larger in some of these animals. Small isolated microconcretions are occasionally observed in the renal tubules of untreated control rats and they are regarded as lesions of naturally occurring disease. In the present study, the formation of such microconcretions appears to have been exacerbated by the test material. The other lesions described in the kidney are those of naturally occurring disease.

D. E. Gordon

Donovan E. Gordon, D. V. M., Ph. D.
Diplomate, American College of
Veterinary Pathologists

TABLE V

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: Control

	An. No. 1	An. No. 2	An. No. 3	An. No. 4	An. No. 5
Kidney		Mc[1]/b	Mc[1]/b, FLI[1]		Mc[1]/b, FLI[1]

TABLE V continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: Control

	An. No. 7	An. No. 8	An. No. 9	An. No. 10	An. No. 11
Kidney		Mc^{3}/b			

Grading System:

+1 = Minimal in severity (less than 10 microconcretions/section)
+2 = Mild in severity (10-30 microconcretions/section)
+3 = Moderate in severity (30-50 microconcretions/section)
+4 = Marked in severity (50-75 microconcretions/section)

Codes:

Mc = Calcified microconcretions in tubules
/b = Bilateral
FLI = Focal lymphoid infiltration

TABLE VI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-I (300 ppm)

	An. No. 46	An. No. 47	An. No. 48	An. No. 49	An. No. 50
Kidney	Mc^{1-2}/b		Mc^{1}/b	Mc^{1}/b	

TABLE VI continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-I (300 ppm)

	An. No. 52	An. No. 53	An. No. 54	An. No. 55	An. No. 56
Kidney	Mc[1]/b, Tc[1], Tn[1], FLI[1]	Mc[1]/u	Mc[1]/b	Mc[1-2]/b	Mc[1]/u, FLI[1]

Grading System:

+1 = Minimal in severity (less than 10 microconcretions/section)
+2 = Mild in severity (10-30 microconcretions/section)
+3 = Moderate in severity (30-50 microconcretions/section)
+4 = Marked in severity (50-75 microconcretions/section)

Codes:

Mc = Calcified microconcretions in tubules
/b = Bilateral
/u = Unilateral
FLI = Focal lymphoid infiltration
Tc = Tubular cast (proteinaceous)
Tn = Tubular nephrosis

TABLE VII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-II (1,000 ppm)

	An. No. 61	An. No. 62	An. No. 63	An. No. 64	An. No. 65
Kidney	Mc[1]/b		Mc[1-2]/b	Mc[1]/b	

TABLE VII continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Histopathologic Findings

Group: T-II (1,000 ppm)

	An. No. 66	An. No. 67	An. No. 68	An. No. 69	An. No. 70
Kidney	Mc^{1}/b, FLI^{1}	Mc^{3}/b	Mc^{1-2}/b	Mc^{1}/u	Mc^{1}/b

Grading System:

+1 = Minimal in severity (less than 10 microconcretions/section)
+2 = Mild in severity (10-30 microconcretions/section)
+3 = Moderate in severity (30-50 microconcretions/section)
+4 = Marked in severity (50-75 microconcretions/section)

Codes:

Mc = Calcified microconcretions in tubules
/b = Bilateral
/u = Unilateral
FLI = Focal lymphoid infiltration

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1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

STAUFFER CHEMICAL COMPANY

EVALUATION OF KIDNEY TISSUES FROM RATS AND DOGS FED KASAL FOR 90 DAYS

FEBRUARY 21, 1974

IBT NO. 661-04633

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STAUFFER CHEMICAL COMPANY

EVALUATION OF KIDNEY TISSUES FROM RATS AND DOGS FED KASAL FOR 90 DAYS

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I. Introduction

This report presents the results of the histopathologic examination of sections of kidneys from rats fed KASAL at dietary levels of 300, 1,000, 3,000 10,000 or 30,000 ppm and from dogs fed 3,000, 10,000 or 30,000 ppm in their diets for 90 days (1, 2 and 3). This comprehensive histopathologic study of rat and dog kidneys was conducted to more thoroughly describe and to evaluate the toxicological significance of the renal microconcretions that were observed in the female rats (1 and 2).

Microconcretions have been observed in the kidneys of rats fed high levels of a number of inorganic phosphate salts (4, 5 and 6); similar microconcretions are also known to occur spontaneously. The highest frequency of spontaneous occurrence of this lesion is found in the female rat; incidences as high as 70% have been observed among untreated female rats in this laboratory. No deleterious effect of these microconcretions on renal function has been determined.

II. Summary

Histopathologic examinations of microconcretions present in the kidneys of female rats fed various dietary levels of KASAL demonstrate that they are identical in appearance to those arising spontaneously among untreated female rats of similar age which have been maintained under identical conditons. These microconcretions are present in the lumina of tubules located primarily at the corticomedullary junction and involve one or both kidneys. Similar microconcretions were occasionally noted in a few tubules located in the cortex and/or medulla. In the initial stage of this lesion, there is focal to diffuse degeneration and eventual necrosis of the tubular epithelium lining isolated tubules which subsequently becomes mineralized. In the later stages of the lesion, there are small concretions within the lumina of affected tubules composed of light to dark blue concentric rings of amorphorus to granular material with a laminated appearance.

There was a greater incidence and an increased number and size of intra-tubular microconcretions in the kidneys of female rats given 10,000 or 30,000 ppm than those of spontaneous origin observed in control rats. Even though the incidences observed in other groups of treated female rats may have been slightly higher than that of their contemporary controls, they were not outside the range observed among control females. No significance is given to the microconcretions found in the female rats fed 300, 1,000 or 3,000 ppm. Renal microconcretions were not present in any of the male rats or in either male or female dogs, even at dietary levels of 3,000, 10,000 or 30,000 ppm.

It is concluded that the female rat is uniquely sensitive to the development of renal microconcretions. Furthermore, the spontaneous incidence of this lesion in the female rat can be increased by feeding high levels of inorganic phosphate. The significance of this finding is highly questionable because it was not associated with any evidence of impaired renal function or any other toxicologic manifestation. Furthermore, neither male rats nor male or female dogs exhibited any evidence of this lesion even at dietary levels as high as 30,000 ppm.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: D. E. Gordon

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Diplomate, American College of
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chm

III. Procedure

A. Experimental Material

The kidney tissues evaluated in this study were from Charles River strain albino rats or purebred beagle dogs utilized in three reported studies (1, 2 and 3). The organization of the individual experiments and the dose used are shown in Table I.

TABLE I

TEST MATERIAL: KASAL

90-Day Subacute Oral Toxicity Study

Outline of Experiments

Experiment Reference	Species	Group	No. of Animals Male	Female	Dietary Level ppm
1	rat	C-I	-	15	none
2	rat	C-II	15	15	none
1	rat	T-I	-	15	300
1	rat	T-II	-	15	1,000
2	rat	T-III	15	15	3,000
2	rat	T-IV	15	15	10,000
2	rat	T-V	15	15	30,000
3	dog	C-III	4	4	none
3	dog	T-VII	4	4	10,000
3	dog	T-VIII	4	4	30,000

B. Histologic Technique

Transverse sections, approximately 4 mm in thickness, were taken from both kidneys of all experimental animals immediately after sacrifice and fixed in 10% neutral buffered formalin. Paraffin-embedded sections of kidney were cut at 4-6 microns, stained with hematoxylin and eosin and examined by light microscopy.

Photomicrographs of representative renal lesions from control and test animals were prepared.

IV. Results

Table II summarizes the results of microscopic examination of the kidney tissue from the control animals and those fed KASAL.

Microconcretions were observed spontaneously only in the female rats. No renal microconcretions were observed in the kidney sections from control or treated male rats or from either male or female dogs of any treatment group. From a review of the data it can be seen that there is a definite treatment and dose related increase in the incidence and relative severity of renal microconcretions among female rats from the higher treatment levels. No treatment related effects were observed in kidney tissue from either the male rats or male or female dogs, even at the highest treatment level.

In the female rats, the microconcretions are present in the lumina of tubules located principally at the corticomedullary junction of one or both kidneys of animals from both the control and treatment groups. The localization of the lesion to tubules in this area of the kidney corresponds to that area where the terminal portion of the proximal convoluted tubules are found. Occasional microconcretions were noted in a few tubules located in the cortex and/or medulla. Various stages were noted in the development of these microconcretions. In the initial stage of their development, there is focal to diffuse degeneration and necrosis of the renal tubular epithelium lining isolated tubules. The cellular debris resulting from the degenerative process appears to serve as a nidus in the initial formation of the typical microconcretion. The cellular debris appears to undergo mineralization (dystrophic calcification) just prior to or shortly after extrusion into the

lumen of affected tubules. Several small microconcretions containing a central nucleoid of mineralization can be identified in some of the affected tubules. As these structures enlarge, the mineralization process proceeds towards the periphery of the debris. Eventually these structures coalesce and completely occlude the tubules with solid masses of calcareous material. There is a complete absence of epithelial cells lining these tubules. Many of these tubules are greatly dilated or distended with the calcereous material. In the end stage of development, the concretions are composed of light to dark blue concentric rings of amorphorus to granular material which has an "onion skin" or laminated appearance. These structures, due to their mineral content, usually shatter upon sectioning of the tissue, which produces tearing and distortion artefacts in the immediate area of the lesion. The kidneys of the test animals from the highest treatment group contain variable numbers of cortical tubules containing atypical regeneration of the tubular epithelium and in some animals, there are foci of chronic inflammation. Some of these lesions were located adjacent to tubules with microconcretions while others occurred distant to the concretions. Scattered foci of interstitial lymphoid infiltrations were also present in the kidney sections of some of the control and test animals. The microconcretions graded as +3 or greater microscopically were clearly evident upon macroscopic examination of hematoxylin and eosin stained sections and appeared as numerous dark foci or a continuous dark band located at the junction of the cortex and the medulla.

The addition of 30,000 ppm (3%) KASAL to the commercial stock added approximately 0.6% available phosphorous to the diet. This amount is equal

to approximately 2 times the amount of available phosphorous normally present in the stock ration fed the rats. Even at this highest level of intake (over 3 times the normal phosphorous requirement) and in those animals with the greatest incidence and severity of kidney microconcretions there was no clinical pathologic evidence of impaired renal function.

There was no significant difference between kidney weights of female rats fed 30,000 ppm KASAL when compared to control animals. There was a small statistically significant difference of kidney to body weight and kidney to brain weight ratios in female rats fed 30,000 ppm KASAL (2).

Renal and liver functions, as measured by various blood and urine parameters (serum alkaline phosphatase, serum glutamic-pyruvic transaminase, serum glutamic oxaloacetic transaminase, blood urea nitrogen, blood glucose, urine glucose, urine albumin, urine pH, urine specific gravity, and urine microparticulates) was normal for all animals examined.

In summary, the typical histologic appearance of the kidneys from the female rats consisted of a dose related increase in the incidence, number and size of intra-tubular microconcretions in various stages of development. The histologic features of this lesion are consistent with those reported in rats following ingestion of excess inorganic phosphate incorporated in the diet (4, 5 and 6). There was no evidence of spontaneous or treatment induced microconcretions in either male rats of male or female dogs, even at dietary levels as high as 30,000 ppm. As a result of these marked sex and species differences in the development of renal microconcretions, the relative significance of this finding in female rats is highly questionable.

TABLE II

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number And Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations in Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium +	Chronic Focal Nephritis +	
Rat	Control I	None	1-F								
			2-F	X		++	+				1
			3-F								
			4-F								
			5-F	X		+	+				
			6-F								
			7-F								
			8-F		X	+++/+++++	+ to +++				
			9-F								
			10-F								
			11-F								
Rat	Control II	None	16-F					+1 (unilateral)			2
			17-F								
			18-F								
			19-F	X		+	+				
			20-F					+1 (unilateral)			
			21-F					+1 (unilateral)			
			23-F								
			24-F								
			26-F								
			27-F								
Rat	Control II		1-M					+1			2
			2-M								
			3-M					+1			
			4-M								
			5-M								
			6-M								
			7-M								
			8-M								
			9-M					+1			
			10-M								

TABLE II continued

TEST MATERIAL: Kasal

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Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number And Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations in Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium +	Chronic Focal Nephritis +	
Rat	T-I	300	46-F	X		+	+				1
			47-F								
			48-F	X		+	+				
			49-F		X	+/+	+				
			50-F								
			52-F								
			53-F								
			54-F								
			55-F		X	+/+	+				
			56-F								
Rat	T-II	1,000	61-F	X		+	+				1
			62-F								
			63-F		X	+/++	+ to ++				
			64-F		X	+/+	+				
			65-F								
			66-F		X	+/+	+				
			67-F		X	+++/++++	+ to ++				
			68-F								
			69-F								
			70-F	X		+	+				
Rat	T-II	3,000	136-F								2
			137-F		X						
			138-F								
			139-F		X						
			140-F	X							
			141-F								
			142-F					+1			
			143-F		X						
			144-F								
			145-F		X						
			146-F								
			147-F		X						
			149-F								
			150-F								

TABLE II continued

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90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number And Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations in Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium +	Chronic Focal Nephritis +	
Rat	T-IV	10,000	166-F								
			167-F								2
			168-F								
			169-F		X	++/++	+ to ++				
			170-F								
			171-F								
			172-F	X		+	+				
			173-F					+1 (bilateral)			
			174-F								
			175-F		X	++/++	+ to +++				
			176-F		X	+/+	+				
			177-F								
			178-F					+1 (unilateral)			
			179-F								
			180-F								
			151-M								2
			152-M								
			153-M								
			154-M		X			+1			
			155-M								
			156-M								
			157-M								
			158-M								
			159-M								
			160-M								

TABLE II continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number And Sex	Unilateral	Bilateral	Microconcretions: Relative Number in each Kidney Section	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations in Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis +	
Rat	T-V	30,000	196-F		X	+++++/+++++	+ to ++++				2
			197-F		X	+++++/+++++	+ to ++++	+1 (bilateral)	+1 (unilateral)		
			198-F		X	++/+++	+ to ++++		+2 (bilateral)		
			199-F		X	+++++/+++++	+ to ++++		+2 (bilateral)		
			200-F		X	+++++/+++++	+ to ++++		+2 (bilateral)		
			201-F		X	+/++	+ to ++		+1 (bilateral)		
			202-F		X	+/+	+	+2 (bilateral)			
			203-F		X	+/++	+		+1 (bilateral)		
			204-F		X	+++++/+++++	+ to ++++		+2 (bilateral)		
			205-F		X	+/+	+ to ++				
			206-F		X	+/+	+				
			207-F		X	+/++	+ to ++	+1 (unilateral)			
			208-F		X	+/+	+				
			209-F		X	++/++	+ to ++				
			210-F	X		+	++				
			181-M					+			2
			182-M								
			183-M								
			184-M								
			185-M								
			186-M								
			187-M								
			188-M								
			189-M								
			190-M								

TABLE II continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number And Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations in Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium +	Chronic Focal Nephritis +	
Dog	UC	None	5-F								3
			6-F								
			7-F								
			8-F								
			1-M								
			2-M								
			3-M								
			4-M								
Dog	T-I	3,000	13-F								3
			14-F								
			15-F								
			16-F								
			9-M								
			10-M								
			11-M					+1			
			12-M								
Dog	T-II	30,000	29-F								3
			30-F								
			31-F								
			32-F		X+3						
			25-M		X+3						
			26-M		X+3						
			27-M								
			28-M					+1			

Symbols

* + = less than 10; ++ = 10-30; +++ = 31-50; ++++ = 51-75; +++++ > 75

X = Present

** + = 2.5-7.5 μ; ++ = 8.0-12 μ; +++ = 13.0-18 μ; ++++ > 18 μ

\+ 1 = trace or minimal in severity; 2 = mild in severity; 3 = moderate in severity; 4 = marked in severity; 5 = extreme in severity

Legends to Figures for Study Number 661-4633

Fig. 1 Section of Kidney fron Control Rat (No. 2) with a few small mineralized microconcretions within tubules located at corticomedullary junction, X12

Fig. 2 Higher magnification of Kidney from control animal in Fig. 1, with partially mineralized microconcretions involving two adjacent tubules, X5

Fig. 3 High magnification of Kidney from animal in Fig. 1, with a large microconcretion in one tubule which is almost completely mineralized, X500

Fig. 4 Section of Kidney from Control Rat (No. 5), with a solitary intra-luminal microconcretion in an early stage of formation which contains a contral nucleoid of mineralization, X500

Fig. 5 Section of Kidney from Control Rat (No. 8), showing numerous mineralized microconcretions, of various sizes, in tubules located at the corticomedullary junction. Note shattering of some of these bodies and distortion of adjacent tubules, X125

Fig. 6 Higher magnification of a microconcretion from animal in Fig. 5, X500

Fig. 7 Section of Kidney from Test Rat (No. 169, Group IV), with multiple mineralized microconcretions within lumina of tubules at corticomedullary junction, X125

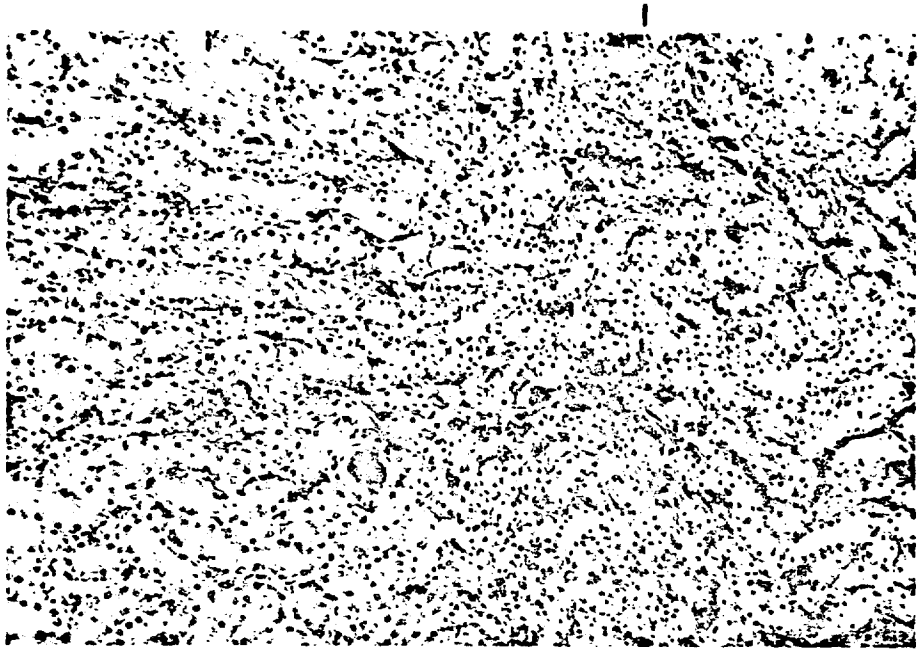
Fig. 8 Higher magnification of one of the microconcretions shown in Fig. 7, X500

Fig. 9 Section of Kidney from Test Rat (No. 175, Group IV), with microconcretions similar to those shown in Fig. 7, X125

Fig. 10 Low magnification of Kidney section from Test Rat (No. 197, Group V), to show the relative number and size of mineralized microconcretions in tubules located principally at the corticomedullary junction. A few small microconcretions can be seen in the lumen of medullary tubules, X 63

Fig. 11 Higher magnification of renal microconcretions from animal in Fig. 10, X125

Fig. 12 Section of Kidney from Test Rat (No. 200, Group V), showing a large mineralized structure, surrounded by tissue in the renal pelvis. Note similar microconcretions in tubules located adjacent to the pelvic epithelium and within the pelvic epithelium, X125



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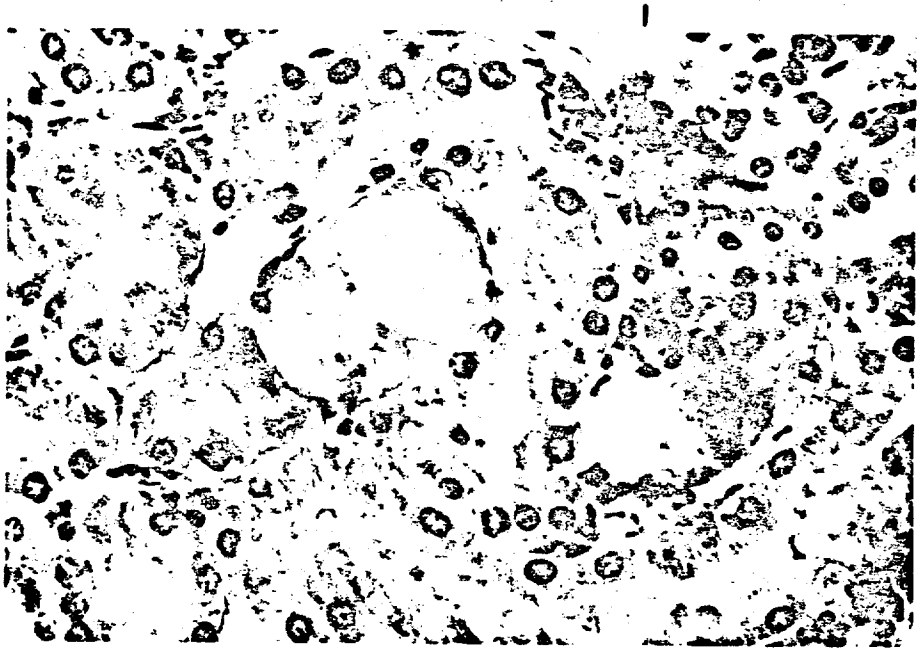

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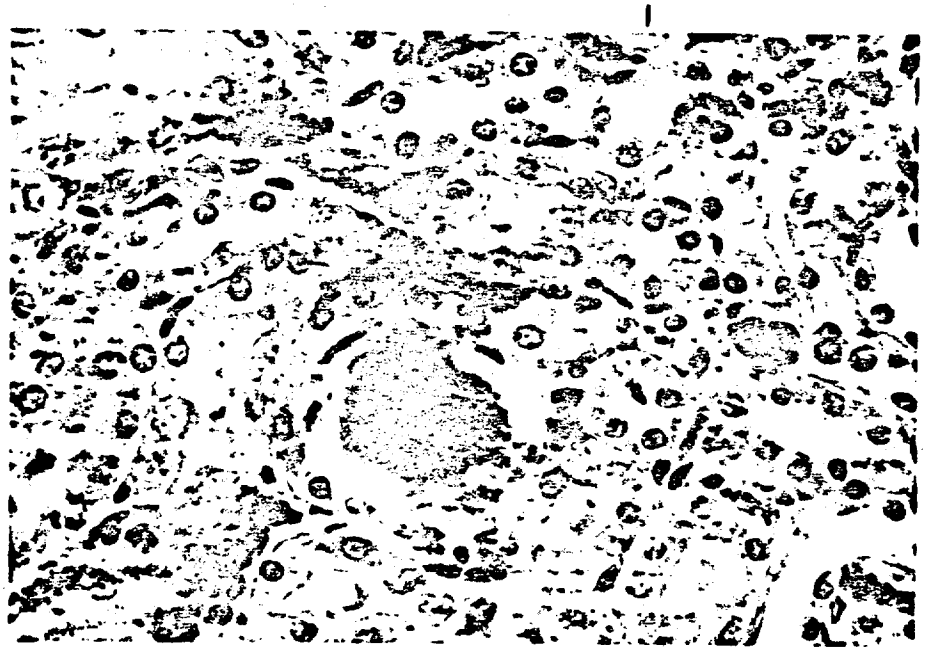

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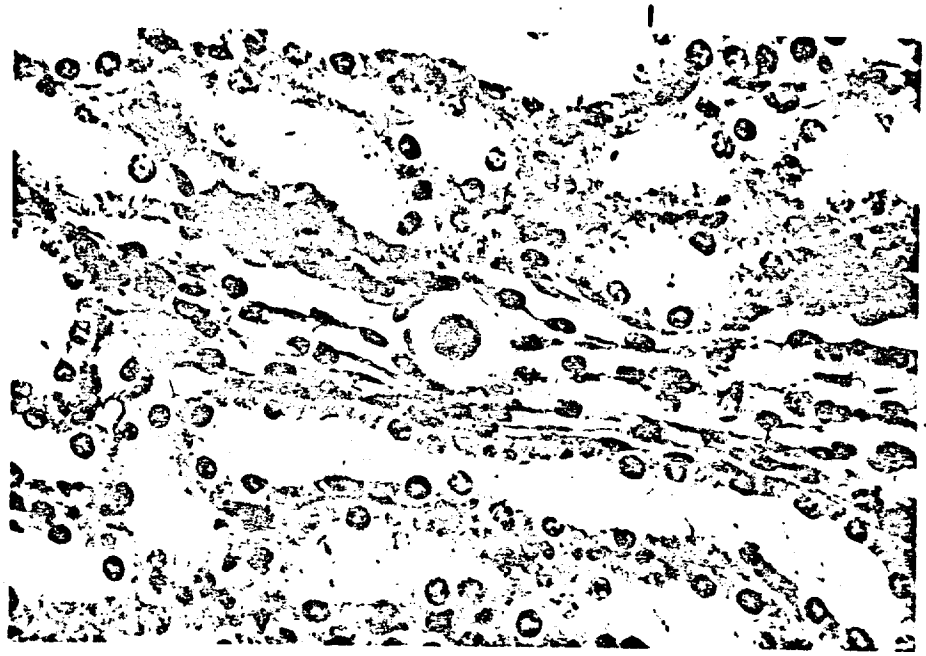

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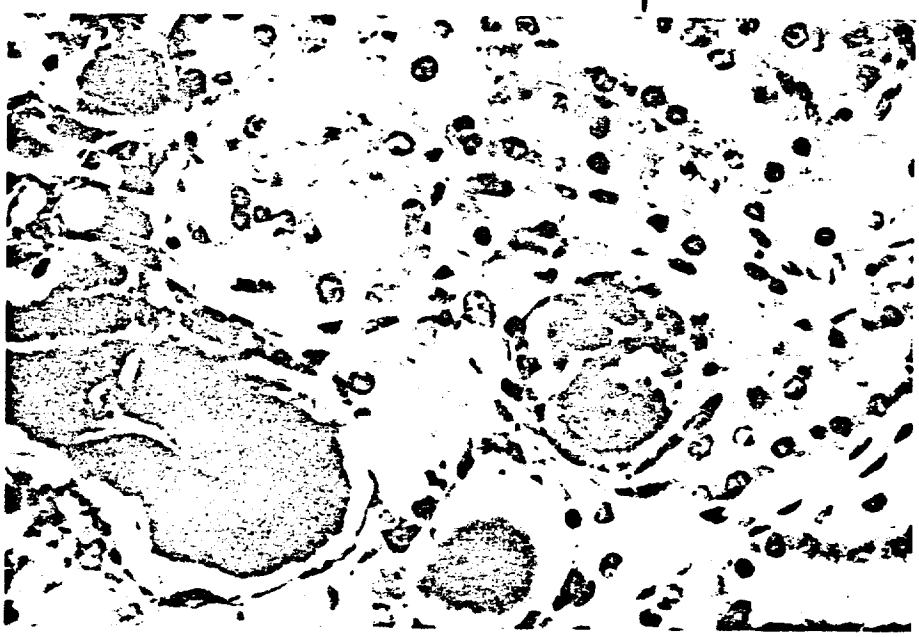

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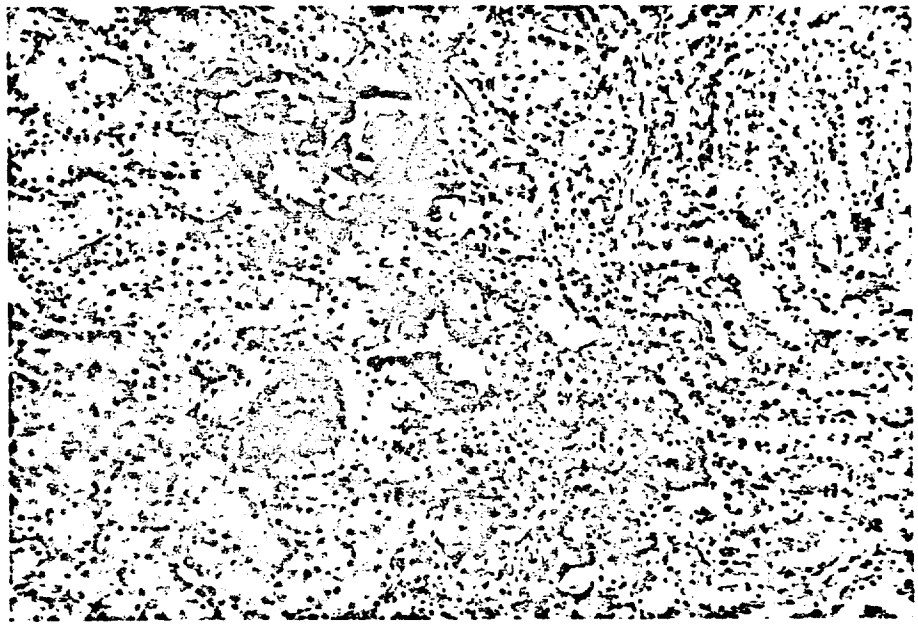

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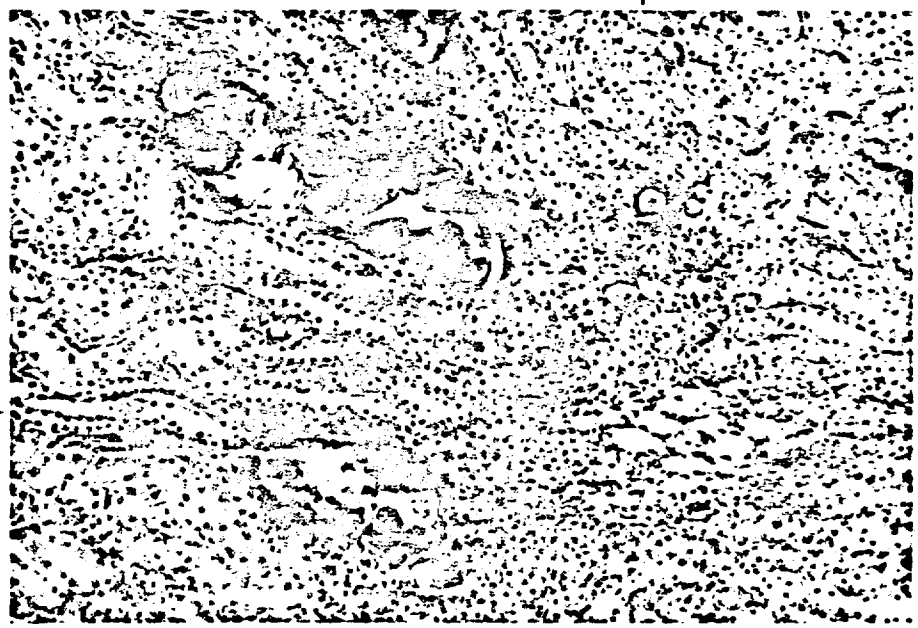

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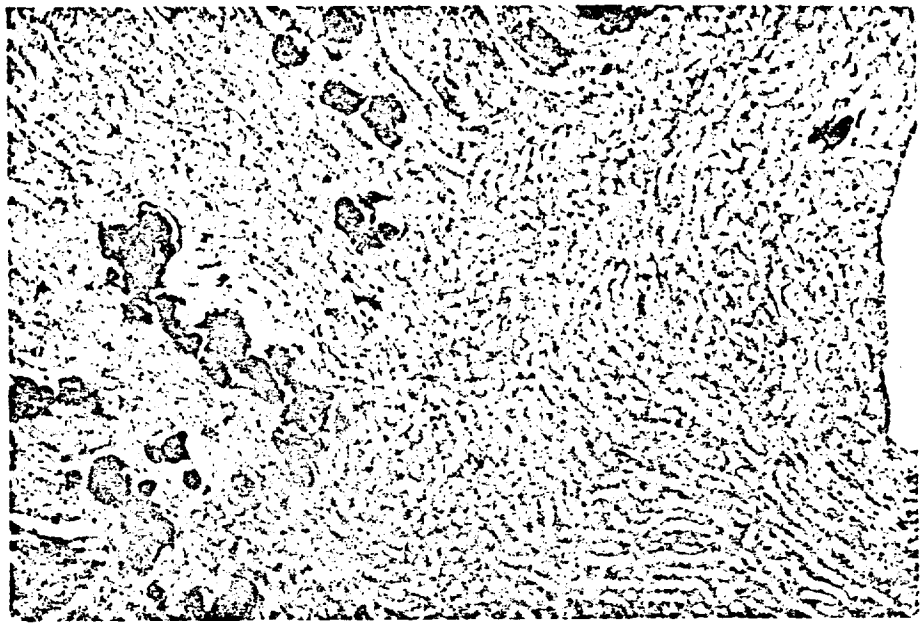

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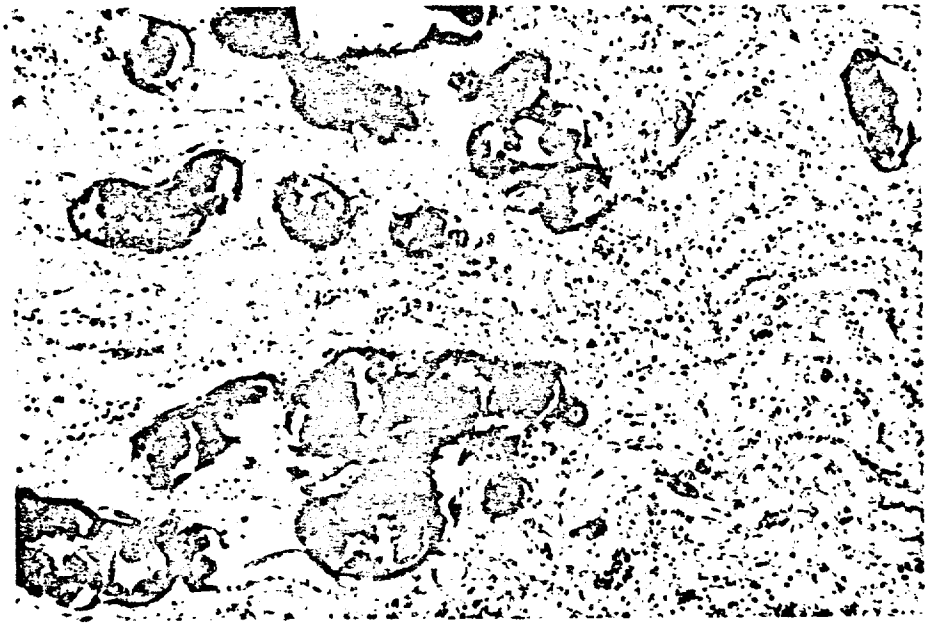

11

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4. Renal Damage Following the Ingestion of a Diet Containing an Excess of Inorganic Phosphate. E. M. McKay and J. Oliver, J. Experimental Medicine 61: 319-333, 1935.

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   II. Chronic Oral Toxicity Studies of Sodium Tripolyphosphate in Rats.

   III. Chronic Oral Toxicity of Sodium Trimetaphosphate in Rats.

   IV. Chronic Oral Toxicity of Sodium Hexametaphosphate in Rats.

   H. C. Hodge, Fd. Cosmet. Toxicology, 2: 147-154, 1964

Industrial **BIO-TEST** *Laboratories, Inc.*
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REPORT TO

STAUFFER CHEMICAL COMPANY

EVALUATION OF KIDNEY TISSUES FROM RATS AND DOGS FED LEVAIR FOR 90 DAYS

FEBRUARY 21, 1974

IBT NO. 661-04633

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

February 21, 1974

Mr. A. B. Lindquist
Stauffer Chemical Company
Nyala Farms Road
West Port, Connecticut 06880

Dear Mr. Lindquist:

Re: IBT No. 661-04633 - Evaluation of Kidney Tissues from Rats and Dogs Fed Levair and Kasal for 90 Days

We are submitting herewith our laboratory reports dated February 21, 1974, prepared in connection with the above study.

Very truly yours,

J. C. Calandra

J. C. Calandra
President

JCC:bp

REPORT TO

STAUFFER CHEMICAL COMPANY

EVALUATION OF KIDNEY TISSUES FROM RATS AND DOGS FED LEVAIR FOR 90 DAYS

FEBRUARY 21, 1974

IBT NO. 661-04633

I. Introduction

This report presents the results of the histopathologic examination of sections of kidneys from rats fed Levair at dietary levels of 300, 1,000, 3,000 10,000 or 30,000 ppm and from dogs fed 3,000, 10,000 or 30,000 ppm in their diets for 90 days (1, 2 and 3). This comprehensive histopathologic study of rat and dog kidneys was conducted to more thoroughly describe and to evaluate the toxicological significance of the renal microconcretions that were observed in the female rats (1 and 2).

Microconcretions have been observed in the kidneys of rats fed high levels of a number of inorganic phosphate salts (4, 5 and 6); similar microconcretions are also known to occur spontaneously. The highest frequency of spontaneous occurrence of this lesion is found in the female rat; incidences as high as 70% have been observed among untreated female rats in this laboratory. No deleterious effect of these microconcretions on renal function has been determined.

II. Summary

Histopathologic examinations of microconcretions present in the kidneys of female rats fed various dietary levels of Levair demonstrate that they are identical in appearance to those arising spontaneously among untreated female rats of similar age which have been maintained under identical conditons. These microconcretions are present in the lumina of tubules located primarily at the corticomedullary junction and involve one or both kidneys. Similar microconcretions were occasionally noted in a few tubules located in the cortex and/or medulla. In the initial stage of this lesion, there is focal to diffuse degeneration and eventual necrosis of the tubular epithelium lining isolated tubules which subsequently becomes mineralized. In the later stages of the lesion, there are small concretions within the lumina of affected tubules composed of light to dark blue concentric rings of amorphorus to granular material with a laminated appearance.

There was a greater incidence and an increased number and size of intra-tubular microconcretions in the kidneys of female rats given 10,000 or 30,000 ppm than those of spontaneous origin observed in control rats. Even though the incidences observed in other groups of treated female rats may have been slightly higher than that of their contemporary controls, they were not outside the range observed among control females. No significance is given to the microconcretions found in the female rats fed 300, 1,000 or 3,000 ppm. Renal microconcretions were not present in any of the male rats or in either male or female dogs, even at dietary levels of 3,000, 10,000 or 30,000 ppm.

It is concluded that the female rat is uniquely sensitive to the development of renal microconcretions. Furthermore, the spontaneous incidence of this lesion in the female rat can be increased by feeding high levels of inorganic phosphate. The significance of this finding is highly questionable because it was not associated with any evidence of impaired renal function or any other toxicologic manifestation. Furthermore, neither male rats nor male or female dogs exhibited any evidence of this lesion even at dietary levels as high as 30,000 ppm.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: D. E. Gordon

Donovan E. Gordon, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists

Report approved by: M. L. Keplinger

M. L. Keplinger, Ph.D.
Manager, Toxicology

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III. Procedure

A. Experimental Material

The kidney tissues evaluated in this study were from Charles River strain albino rats or purebred beagle dogs utilized in three reported studies (1, 2 and 3). The organization of the individual experiments and the dose used are shown in Table I.

TABLE I

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study

Outline of Experiments

Experiment Reference	Species	Group	No. of Animals Male	Female	Dietary Level ppm
1	rat	C-I	-	15	none
2	rat	C-II	15	15	none
1	rat	T-I	-	15	300
1	rat	T-II	-	15	1,000
2	rat	T-III	15	15	3,000
2	rat	T-IV	15	15	10,000
2	rat	T-V	15	15	30,000
3	dog	C-III	4	4	none
3	dog	T-VII	4	4	10,000
3	dog	T-VIII	4	4	30,000

B. Histologic Technique

Transverse sections, approximately 4 mm in thickness, were taken from both kidneys of all experimental animals immediately after sacrifice and fixed in 10% neutral buffered formalin. Paraffin-embedded sections of kidney were cut at 4-6 microns, stained with hematoxylin and eosin and examined by light microscopy.

Photomicrographs of representative renal lesions from control and test animals were prepared.

IV. Results

Table II summarizes the results of microscopic examination of the kidney tissue from the control animals and those fed Levair.

Microconcretions were observed spontaneously only in the female rats. No renal microconcretions were observed in the kidney sections from control or treated male rats or from either male or female dogs of any treatment group. From a review of the data it can be seen that there is a definite treatment and dose related increase in the incidence and relative severity of renal microconcretions among female rats from the higher treatment levels. No treatment related effects were observed in kidney tissue from either the male rats or male or female dogs, even at the highest treatment level.

In the female rats, the microconcretions are present in the lumina of tubules located principally at the corticomedullary junction of one or both kidneys of animals from both the control and treatment groups. The localization of the lesion to tubules in this area of the kidney corresponds to that area where the terminal portion of the proximal convoluted tubules are found. Occasional microconcretions were noted in a few tubules located in the cortex and/or medulla. Various stages were noted in the development of these microconcretions. In the initial stage of their development, there is focal to diffuse degeneration and necrosis of the renal tubular epithelium lining isolated tubules. The cellular debris resulting from the degenerative process appears to serve as a nidus in the initial formation of the typical microconcretion. The cellular debris appears to undergo mineralization (dystrophic calcification) just prior to or shortly after extrusion into the

lumen of affected tubules. Several small microconcretions containing a central nucleoid of mineralization can be identified in some of the affected tubules. As these structures enlarge, the mineralization process proceeds towards the periphery of the debri. Eventually these structures coalesce and completely occlude the tubules with solid masses of calcareous material. There is a complete absence of epithelial cells lining these tubules. Many of these tubules are greatly dilated or distended with the calcereous material. In the end stage of development, the concretions are composed of light to dark blue concentric rings of amorphorus to granular material which has an "onion skin" or laminated appearance. These structures, due to their mineral content, usually shatter upon sectioning of the tissue, which produces tearing and distortion artefacts in the immediate area of the lesion. The kidneys of the test animals from the highest treatment group contain variable numbers of cortical tubules containing atypical regeneration of the tubular epithelium and in some animals, there are foci of chronic inflammation. Some of these lesions were located adjacent to tubules with microconcretions while others occurred distant to the concretions. Scattered foci of interstitial lymphoid infiltrations were also present in the kidney sections of some of the control and test animals. The microconcretions graded as +3 or greater microscopically were clearly evident upon macroscopic examination of hematoxylin and eosin stained sections and appeared as numerous dark foci or a continuous dark band located at the junction of the cortex and the medulla.

The addition of 30,000 ppm (3%) Levair to the commercial stock added approximately 0.8% available phosphorous to the diet. This amount is equal

to slightly over 2 times the amount of available phosphorus normally present in the stock ration fed the rats. Even at this highest level of intake (over 3 times the normal phosphorous requirement) and in those animals with the greatest incidence and severity of kidney microconcretions there was no clinical pathologic evidence of impaired renal function. There were no statistically significant differences in kidney weights, kidney to body weight ration, and kidney to brain weight ratio in female rats fed 30,000 ppm Levair when compared to those of control animals.

Renal and liver functions, as measured by various blood and urine parameters (serum alkaline phosphatase, serum glutamic-pyruvic transaminase, serum glutamic oxaloacetic transaminase, urine albumin, urine pH, urine specific gravity, and urine microparticulates) was normal for all animals examined.

In summary, the typical histologic appearance of the kidneys from the female rats consisted of a dose related increase in the incidence, number, and size of intra-tubular microconcretions in various stages of development. The histologic features of this lesion are consistent with those reported in rats following ingestion of excess inorganic phosphate incorporated in the diet (4, 5 and 6). There was no evidence of spontaneous or treatment induced microconcretions in either male rats or male or female dogs, even at dietary levels as high as 30,000 ppm. As a result of the marked sex and species differences in the development of renal microconcretions, the relative significance of this finding in female rats is highly questionable.

TABLE II

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	C-I	None	1-F								1
			2-F	X		++	+				
			3-F								
			4-F								
			5-F	X		+	+				
			7-F								
			8-F		X	+++/+++++	+ to +++				
			9-F								
			10-F								
			11-F								
	C-II	None	16-F					+1 (unilateral)			2
			17-F								
			18-F								
			19-F	X		+	+				
			20-F					+1 (unilateral)			
			21-F					+1 (unilateral)			
			23-F								
			24-F								
			26-F								
			27-F								
Rat	C-II	None	1-M					+1			2
			2-M					+1			
			3-M								
			4-M								
			5-M								
			6-M								
			7-M								
			8-M								
			9-M					+1			
			10-M								

TABLE II continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	T-I	300	16-F								1
			17-F								
			18-F	X		+	+				
			19-F								
			20-F								
			21-F	X		+	+ to ++				
			22-F		X	++/+++	+ to ++				
			23-F		X	+/+	+ to ++				
			24-F	X		+	+				
			27-F	X		+	+				
Rat	T-II	1,000	31-F		X	+/+++	+ to ++				1
			32-F		X	+/++	+ to ++				
			33-F	X		+	+				
			34-F								
			35-F		X	+/++	+ to ++				
			36-F	X		+	+				
			37-F								
			38-F								
			39-F								
			40-F								
Rat	T-III	3,000	46-F								2
			47-F	X					+1 (unilateral)		
			48-F								
			49-F								
			50-F								
			51-F								
			52-F	X							
			53-F								
			54-F								
			55-F								
			56-F								
			57-F		X					+2	
			58-F								
			59-F								
			60-F								

TABLE II continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	T-IV	10,000	76-F	X		++	+ to ++	+1 (bilateral)			2
			77-F								
			78-F		X	+/+	+				
			79-F		X	+/+	+				
			80-F	X		+	+				
			81-F						+1 (bilateral)		
			82-F		X	+/++	+ to ++				
			83-F								
			84-F		X	+/++	+ to ++				
			85-F								
			86-F		X	+/+	+				
			87-F		X	+/+	+				
			88-F		X	+/+	+	+1 (bilateral)			
			89-F					+1 (bilateral)			
			90-F		X	+/+	+				
			61-M								
			62-M								
			63-M								
			64-M								2
			65-M								
			66-M						+1		
			67-M								
			68-M								
			69-M								
			70-M								

TABLE II continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	T-V	30,000	101-F								2
			102-F								
			103-F								
			104-F								
			105-F	X		+	+				
			106-F								
			107-F		X	+/++	+ to ++++				
			108-F		X	+++/+++++	+ to +++	+1 (bilateral)			
			109-F								
			110-F		X	++/++	+ to +++	+1 to +2 (bilateral)	+1 (bilateral)		
			112-F		X	+++++/+++	+ to ++++		+1 (bilateral)		
			113-F		X	++/++	+ to +++				
			115-F		X	+/+++	+ to +++				
			116-F		X	+++/+++	+ to +++				
			117-F		X	+++/++++	+ to ++++	+1 to +2 (bilateral)	+2 (bilateral)		
			91-M								2
			92-M								
			93-M								
			94-M								
			95-M								
			96-M								
			97-M							X	
			98-M								
			99-M								
			100-M								

TABLE II continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Dog	UC	None	5-F								3
			6-F								
			7-F								
			8-F								
			1-M								
			2-M								
			3-M								
			4-M								
Dog	T-I	3,000	13-F								3
			14-F								
			15-F								
			16-F								
			9-M								
			10-M								
			11-M					+1			
			12-M								
Dog	T-II	10,000	21-F								3
			22-F								
			23-F								
			24-F								
			17-M								
			18-M								
			19-M								
			20-M								

TABLE II continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Dog	T-III	30,000	29-F								3
			30-F								
			31-F								
			32-F								
			25-M								
			26-M								
			27-M								
			28-M								

Symbols

* + = less than 10; ++ = 10-30; +++ = 31-50; ++++ = 51-75; +++++ > 75

** + = 2.5-7.5 μ; ++ = 8.0-12 μ; +++ = 13.0-18 μ; ++++ > 18 μ

\+ 1 = trace or minimal in severity; 2 = mild in severity; 3 = moderate in severity; 4 = marked in severity; 5 = extreme in severity

X = Present

Legends to Figures for Study Number 661-4633

Fig. 1 Section of Kidney fron Control Rat (No. 2) with a few small mineralized microconcretions within tubules located at corticomedullary junction, X125

Fig. 2 Higher magnification of Kidney from control animal in Fig. 1, with partially mineralized microconcretions involving two adjacent tubules, X500

Fig. 3 High magnification of Kidney from animal in Fig. 1, with a large microconcretion in one tubule which is almost completely mineralized, X500

Fig. 4 Section of Kidney from Control Rat (No. 5), with a solitary intra-luminal microconcretion in an early stage of formation which contains a central nucleoid of mineralization, X500

Fig. 5 Section of Kidney from Control Rat (No. 8), showing numerous mineralized microconcretions, of various sizes, in tubules located at the corticomedullary junction. Note shattering of some of these bodies and distortion of adjacent tubules, X125

Fig. 6 Higher magnification of a microconcretion from animal in Fig. 5, X500

Fig. 7 Section of Kidney from Test Rat (No. 82, Group IV), with multiple mineralized microconcretions within lumina of tubules located at corticomedullary junction, X125

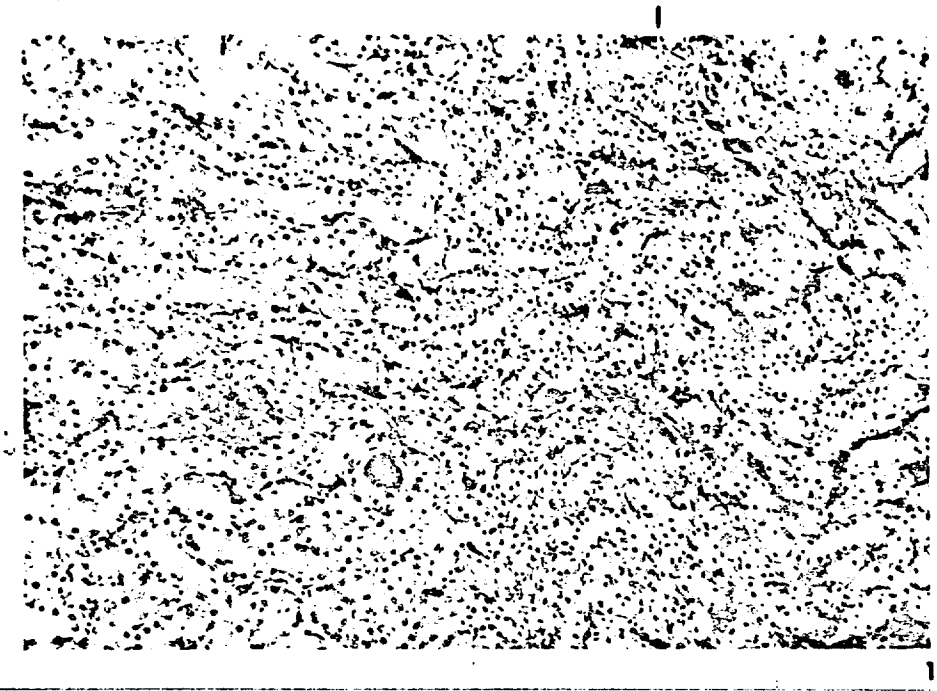
Fig. 8 Higher magnification of one of the microconcretions shown in Fig. 7, X500

Fig. 9 Section of Kidney from Test Rat (No. 84, Group IV), with microconcretions similar in appearance and location to those shown in Fig. 7, X125

Fig. 10 Section of Kidney from Test Rat (No. 107, Group IV), with a large mineralized microconcretion which completely occludes the lumen of the affected tubule. The tissue surrounding this lesion is normal in appearance, X125

Fig. 11 Section of Kidney from Test Rat (No. 108, Group V), showing microconcretions in various stages of formation, and size. There is early mineralization of degenerate cells and cellular debris which have been sloughed into the lumen of the large tubules in the center of the field. Degenerate epithelial cells are also evident in some of the adjacent tubules. The microconcretion seen in the upper right corner of the photograph is in a more advanced stage of development, X500

Fig. 12 Section of Kidney from Test Rat (No. 115, Group V), showing a cluster of adjacent tubules located at the corticomedullary junction which contain mineralized microconcretions of various sizes. Some of the affected tubules are completely occluded and distended with these bodies, X125



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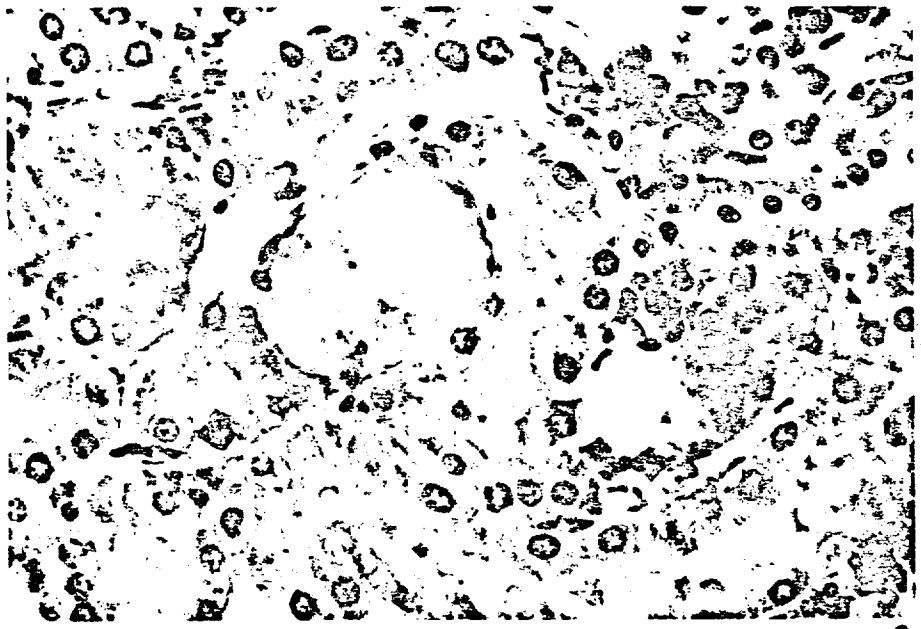

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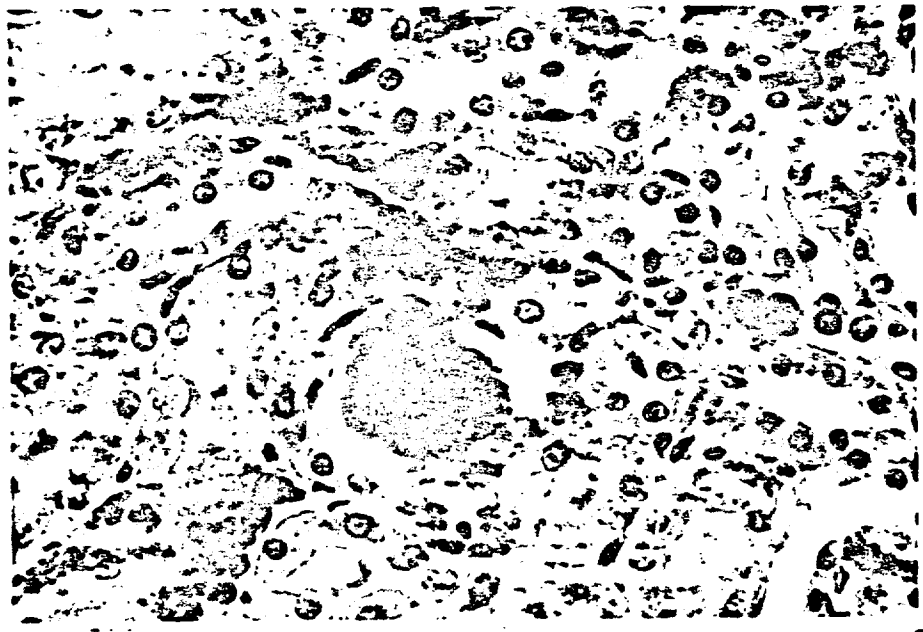

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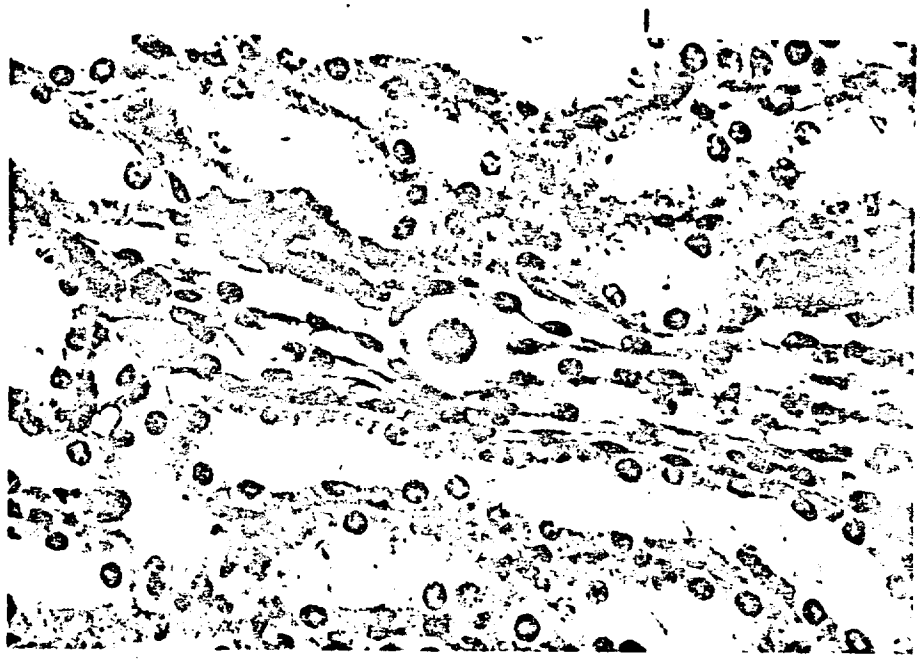

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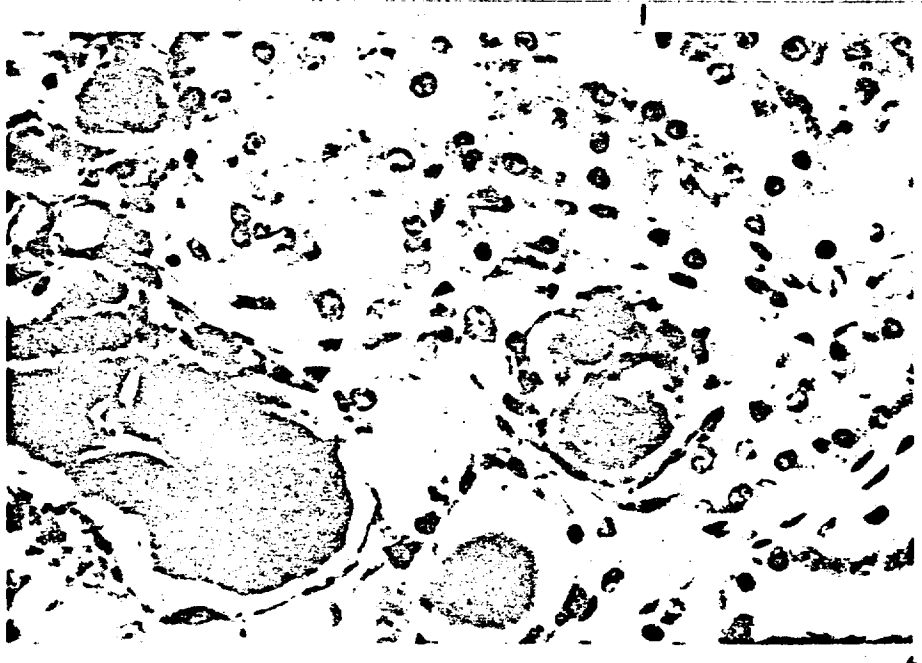

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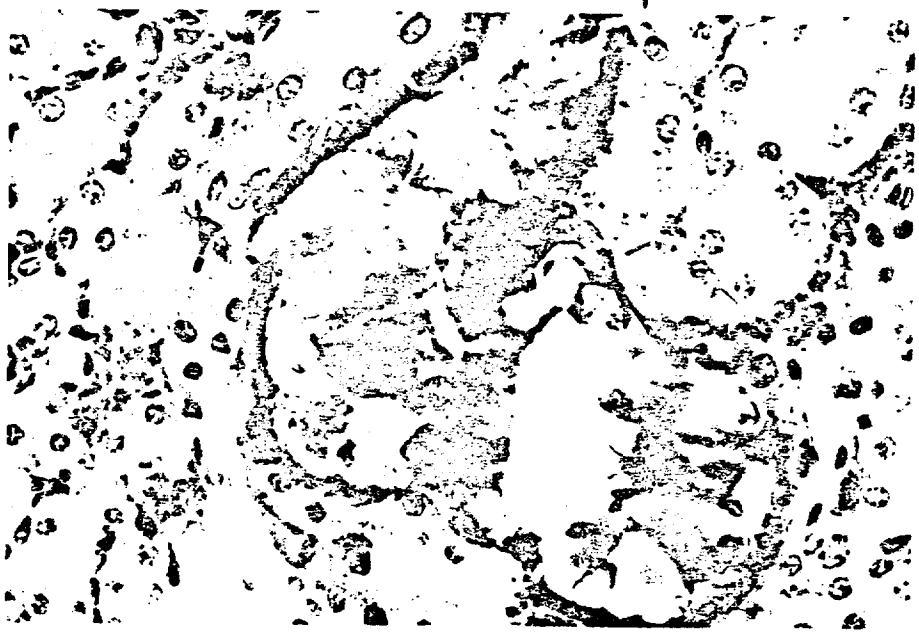

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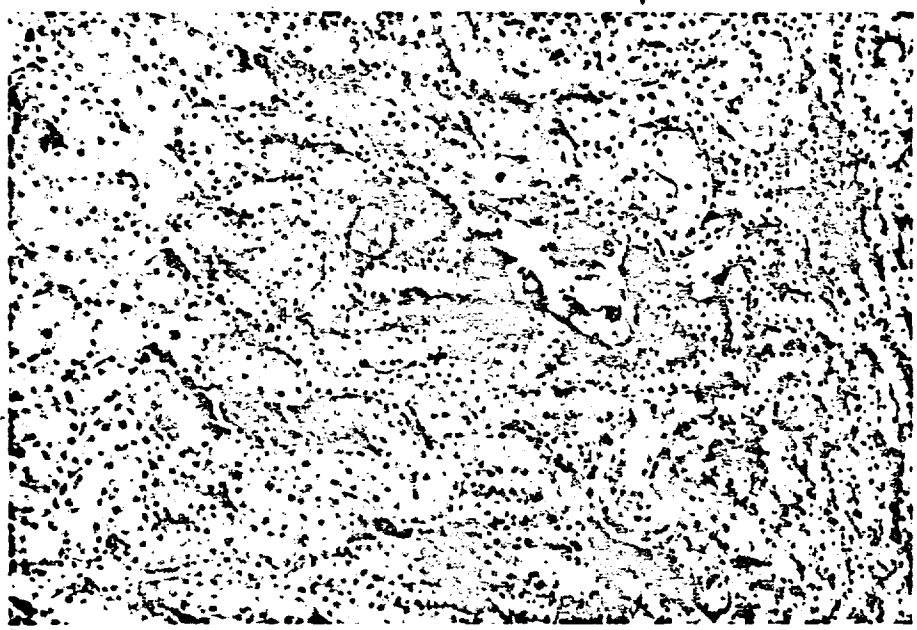

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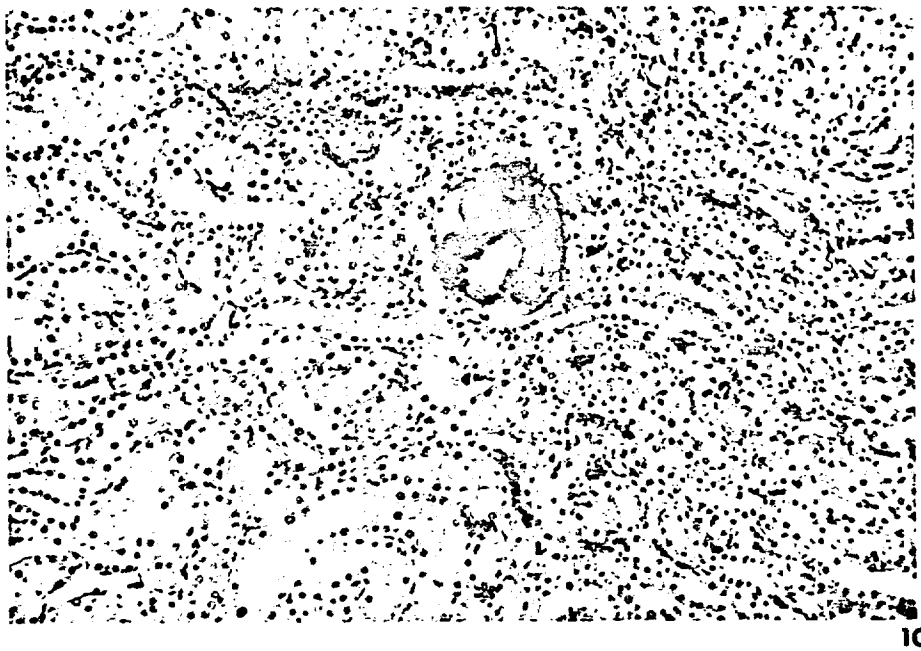

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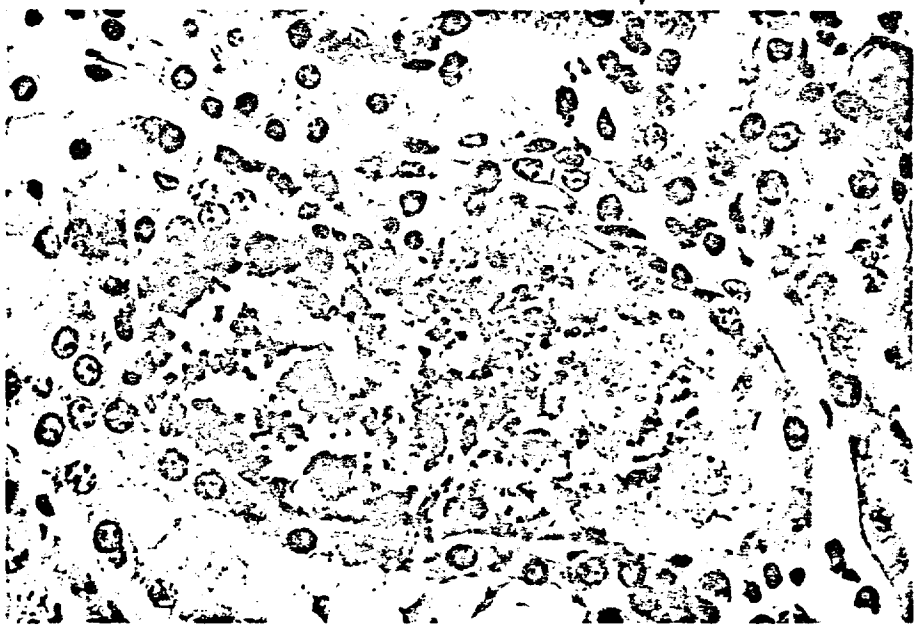

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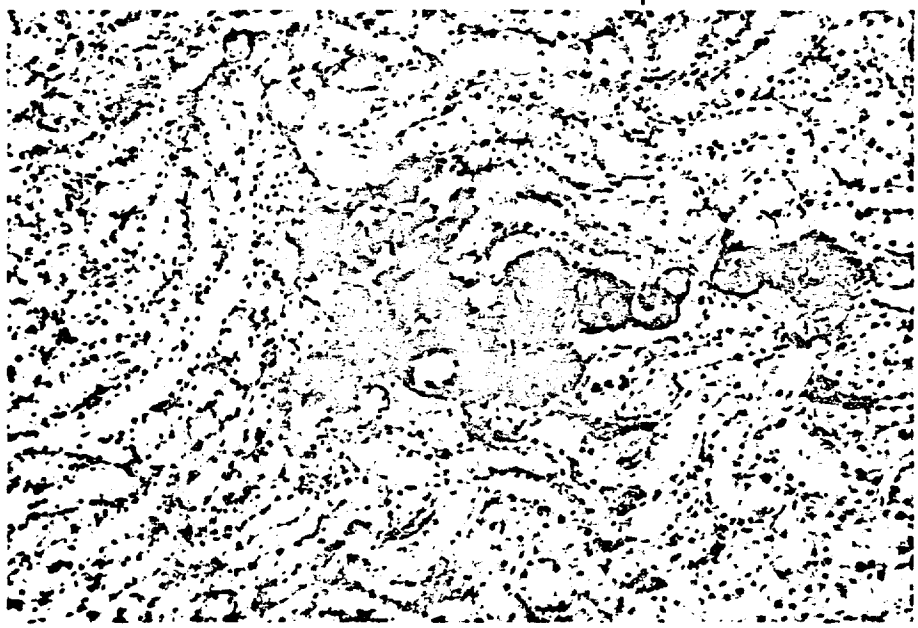

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